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Sources and fate of dissolved free amino acids in epilimnetic Lake Michigan water[1]

Abstract—A seasonal series of light- and dark-bottle experiments was conducted on epilimnetic Lake Michigan water during 1986 to examine the dynamics of dissolved free amino acids (DFAA) in the pelagic microbial food web. When microbial uptake sites were saturated with DFAA, net primary amine removal rates were lower in light than in dark bottles, presumably due to release of DFAA by phytoplankton. Release rates ranged from 0.8 to 3.5 ng-atoms N liter^{-1} h^{-1} in bottled lake water held several days at in situ temperatures. Ammonium accumulation rates in the dark ranged from <0.4 to 4 nmol liter^{-1} h^{-1}. Ammonium regeneration was enhanced (up to 3–8 nmol liter^{-1} h^{-1}) by the addition of DFAA (0.94 μM) to unfiltered lake water. On average, the increases in ammonium-N accumulation equaled losses of amino acid nitrogen from the same water samples [ratio = 1.01 (SE = 0.08, n = 29)]. This result suggests that Lake Michigan microheterotrophs may deaminate DFAA and not maintain the nitrogen in food-web biomass.

The importance of the "microbial food web" to energy flow and nutrient cycling processes in pelagic zones of lakes and other aquatic systems is widely recognized (e.g. Sherr et al. 1983; Sieburth 1984; Caron et al. 1985; Fahnenstiel et al. 1986; Stockner and Antia 1986), but details of nutrient transformations by the various components of the food web are still unclear. Modes of transfer of photosynthesized nutrients from phytoplankton to free-living bacteria (Azam and Ammerman 1984; Cole et al. 1982; Coveney 1982) and other heterotrophs and the relative importance of the various heterotrophs in making inorganic nutrients available to phytoplankton are only partially understood.

Release rates of specific classes of labile materials (e.g. amino acids, organic acids, and carbohydrates) must be determined to assess the importance of these compounds in the link between autotrophic and heterotrophic production. Release rates are difficult to measure because bacteria tend to remove labile compounds at about the rate that they are normally supplied (Iturriaga and Zsolnay 1983). Thus, although concentrations of these compounds may be low or undetectable in natural waters, their flux may be significant (Fuhrman 1987). For example, qualitative changes in the C:N ratios of dissolved free amino acids (DFAA) in the North Sea during a phytoplankton bloom indicated a greater DFAA turnover than was suggested from the examination of trends in DFAA concentration patterns alone (Hammer and Kattner 1986).

Differentiation of nutrient production from uptake processes in the microbial food web depends on separating the effects of primary production from those of mineralization without substantially affecting community dynamics (Iturriaga and Zsolnay 1981). Differential filtration is not completely satisfactory because the two groups often overlap in size (Fuhrman and McManus 1984; Rassoulzadegan and Sheldon 1986), some organisms function as mixotrophs (Bird and Kalff 1986), and indirect interactions between autotrophs and heterotrophs (e.g. nutrient exchange) may be modified by physically separating the groups (Sherr et al. 1986). Selective inhibition by antibiotics can cause partial lysis of affected organisms (Sherr et al. 1986) and may have side effects on nontarget organisms (Jensen 1983, 1984). These problems have recently been avoided in measurements of amino acid uptake and release in seawater by using isotope dilution techniques in conjunction with high performance liquid chromatography (Fuhrman 1987).

Comparative kinetic studies of natural and added concentrations of amino acids and ammonium in lake water held in "dark" and "light/dark" bottles offer another ap-

[1] GLERL Contribution 555.

Table 1. Rates of amino acid release (ng-atoms N liter^{-1} h^{-1}) by phytoplankton in epilimnetic water of Lake Michigan sampled during 1986. Rates of primary amine release were calculated as differences in primary amine concentrations between light and dark bottles over time after addition of an amino acid mixture (0.94 μM) to each to saturate microbial uptake sites (except for the sample of 23 April that had a high initial concentration of primary amines). Amino acid concentrations were assumed to be 1.46 times PA concentrations. Number of data sets compared per regression—*n*; correlation coefficient—*r*. Data for the 9 July water sample (23°C) were not collected frequently enough for accurate calculation of PA removal rates and are not presented.

1986	Temp (°C)	*n*	Untreated		1 μM NH_4^+ addition		3-μm filtrate		0.8-μm filtrate	
			Rate	*r*	Rate	*r*	Rate	*r*	Rate	*r*
23 Apr	3	8	1.2	0.95	1.6	0.96	1.6	0.89	0.3	0.55
7 May	7	7	2.0	0.98	2.8	0.98	0.4	0.90	0.0	0.30
22 May	6	9	0.8	0.85	1.2	0.90	0.2	0.68	0.2	−0.31
9 Jun	13	8	2.4	0.84	1.7	0.98	−0.1	−0.17	−0.2	−0.47
28 Jul	17	4	1.4	0.99	6.3	0.94	0.2	0.67	0.7	0.26
20 Aug	18	3	3.5	1.00	0	0	0.06	0.50	0.8	0.61
23 Sep	17	5	1.2	0.97	0.6	0.88	0.7	0.84	0.04	0.03
26 Nov	8	9	1.6	0.91	1.1	0.90	0.91	0.87	0.18	0.71

proach to examine autotrophic-heterotrophic interactions with respect to dissolved nitrogen compounds. In this paper, we chemically examine production and uptake of dissolved nitrogen (ammonium and amino acids) over several days in bottles of unfortified and amino acid-fortified epilimnetic Lake Michigan water and examine the fate (food-web biomass vs. mineralization) of DFAA nitrogen. Lake Michigan provides an interesting environment in which to examine these phenomena, compared to marine and eutrophic lake systems, because it is a relatively oligotrophic system where the phytoplankton are not under nitrogen limitation.

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Epilimnetic water was collected from a depth of 3–4 m by Niskin bottle at a 45-m-deep station, offshore from Grand Haven, Michigan, periodically between April and November 1986. The lake water was placed in clean LPE jugs and stored at approximately in situ temperatures (Table 1). Experiments were begun within 24 h of sample collection. Subsamples of lake water were preserved with Lugol's solution on most sampling dates for later preparation of phytoplankton slides (Dozier and Richerson 1975). For the June sample, the contents of the light and dark bottles of unfiltered lake water (*see below*) were also preserved and examined for phytoplankton content at the end of a 13-d incubation for comparison with the initial sample. Samples for bacterial counts were preserved at the beginning and end of experiments with ~1% Formalin. Bacterial abundance was determined by the acridine orange direct-count method (Hobbie et al. 1977).

For each light–dark experiment, replicate 70-ml portions of lake water were subjected to a series of pretreatments, including ammonium addition (1 μM) and filtration through 0.8- and 3-μm pore-size filters, and poured into sterile 70-ml tissue culture bottles (Corning tissue culture flasks, 25 cm^2 size). The 0.8-μm pore-size filters were Gelman Metricel membrane filters (Prod. No. 60109) (April and May) or Nuclepore polycarbonate filters (SN 110609) (June through November). The 3-μm filters were Nuclepore Membra-Fil membrane filters (SN 140612) (April to August) or Nuclepore polycarbonate filters (SN 110612) (September to November). Samples were filtered directly into the tissue culture bottles with a clean 60-ml syringe attached to a clean 25-mm-diameter filter holder. The first 40 ml of sample filtrate was discarded to prevent contamination from the filtration apparatus. Comparison of untreated and prefiltered samples indicated that filtration had no effect on concentrations of measurable primary amines or ammonium in the water.

Two of the four bottles from each treatment were fortified with an amino acid mix-

ture containing 3.75 nmol each of NH_4^+ and the following amino acids: L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, L-valine, and 1.87 nmol of L-cystine in 0.1 M HCl (mixture AA-S-18, Sigma Chemical Co.). It resulted in a total addition of 0.94 μmol amino acid (0.64 μmol of measurable primary amines) per liter of lake water. This concentration, chosen to saturate microbial uptake sites, was based on previous data on Lake Michigan water (Gardner et al. 1986). One bottle of each treatment was incubated in the dark (dark bottle) at in situ temperature; the other bottle (light bottle) was held under 12 : 12 L/D conditions.

Each treatment bottle was gently mixed at 1–3-d intervals and a 1-ml sample was removed (with a prerinsed needle and syringe) and analyzed for ammonium and "primary amines" (PA) by a previously described technique for sampling and discrete-injection liquid chromatographic measurement (Gardner et al. 1986). PA is defined as being amino acids (and possibly other primary amines) less basic than ammonium that form fluorescent derivatives with *o*-phthalaldehyde. In this technique, a water sample is filtered (Gardner and Vanderploeg 1982) and injected directly into a chromatographic analyzer that separates the PA (as a group) from ammonium and quantifies both peaks as fluorescent derivatives of *o*-phthalaldehyde (OPA; Gardner 1978; Gardner and Miller 1981). Concentrations of total amino acid nitrogen were calculated from measurements of PA nitrogen with a factor of 1.46 to account for amino acids not measured by the chromatographic technique. This factor was the molar ratio of amino acid nitrogen in the added mixture to the nitrogen in the OPA-fluorescing amino acid peak that eluted before ammonium on the analyzer (Gardner et al. 1986). For calculations of unknown amino acids released by phytoplankton, we arbitrarily assumed that the ratio of PA to DFAA in the samples was the same as in the standard mixture.

"Amino acid release rates" were estimated from the linear least-squares slope of $(1.46) \times (PA_L - PA_D)$ vs. time (where 1.46 is the conversion factor and PA_L and PA_D are PA concentrations in corresponding light and dark bottles). Dark-bottle concentrations were subtracted from light-bottle concentrations at each sampling point to correct both for microbial uptake of amino acids and for possible autolytic release of amino acids caused by phytoplankton death due to containment. The slopes were calculated for the period beginning when the light-bottle and dark-bottle curves began to diverge (1–3 d after incubations began) and ending either when the experiment was stopped or when PA concentrations in one of the bottles reached background levels.

Phytoplankton composition at the study site before experimentation exhibited typical (Fahnenstiel and Scavia 1987*b*) seasonal variation. The spring phytoplankton assemblage was dominated by diatoms and cryptophytes. During thermal stratification, cryptophytes, blue-green algae, and smaller unidentified flagellates became more abundant, while diatom numbers declined. During fall isothermal conditions, the assemblage was again dominated by cryptophytes and several species of diatoms.

Results from extended bottle experiments cannot be directly extrapolated to natural conditions because phytoplankton (and other organisms) can change in quantity and composition during containment (Venrick et al. 1977). We examined phytoplankton changes in the 9 June experiment. The contents of the light and dark bottles of unfiltered lake water were preserved and examined for phytoplankton content at the end of the 13-d incubation and compared to results for the initial water sample. Incubation in the 70-ml tissue-culture bottles under dark and light/dark conditions had discernible effects on phytoplankton biomass and composition. Before incubation, the lake water had a typical (Fahnenstiel and Scavia 1987*b*) spring phytoplankton composition assemblage dominated by cryptomonads (48.2%) and diatoms (39.8%). The total phytoplankton carbon content in the original sample was 91 mg C m^{-3}. After the 13-d incubation, the phytoplankton biomass in the dark water sample decreased to 65 mg C m^{-3} and was dominated by diatoms (56.1%), cryptomonads (24.4%), and

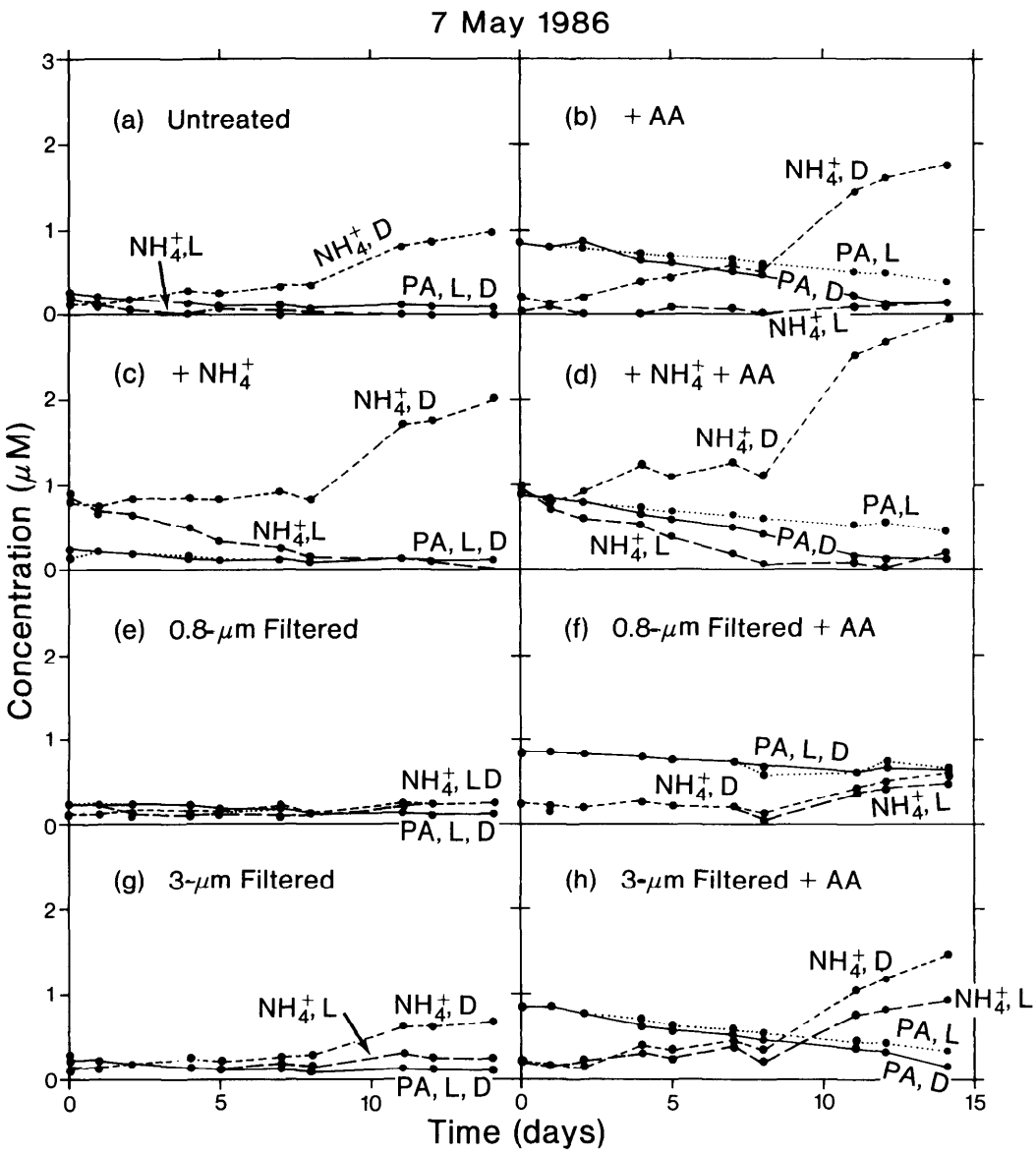

FIG. 1. Patterns of ammonium and primary amine concentrations vs. time in 12:12 L/D and dark bottles of Lake Michigan water sampled on 7 May 1986 and held in 70-ml tissue culture bottles at in situ temperature of 7°C for 14 d. Ammonium chloride (1 μM) was added to the bottles labeled "+ NH_4^+." Dissolved free amino acids (0.94 μM) were added to the "+ AA" treatments. PA—Operationally defined primary amines; D—dark conditions; L—12:12 L/D conditions.

blue-green algae (15.5%). In contrast, the biomass of the light sample increased to 264 mg C m^{-3} with high abundances of chrysophytes (36.1%), diatoms (28.9%), and blue-green algae (13.0%). In addition to phytoplankton, both dark and light samples had increased populations of unidentified heterotrophic protozoans (dark, 26.7 mg C m^{-3}; light, 184 mg C m^{-3}).

We did not investigate the reasons for the increases in phytoplankton and protozoan biomass during the light incubations. Increased nutrient supply seems unlikely because great care was taken to avoid contam-

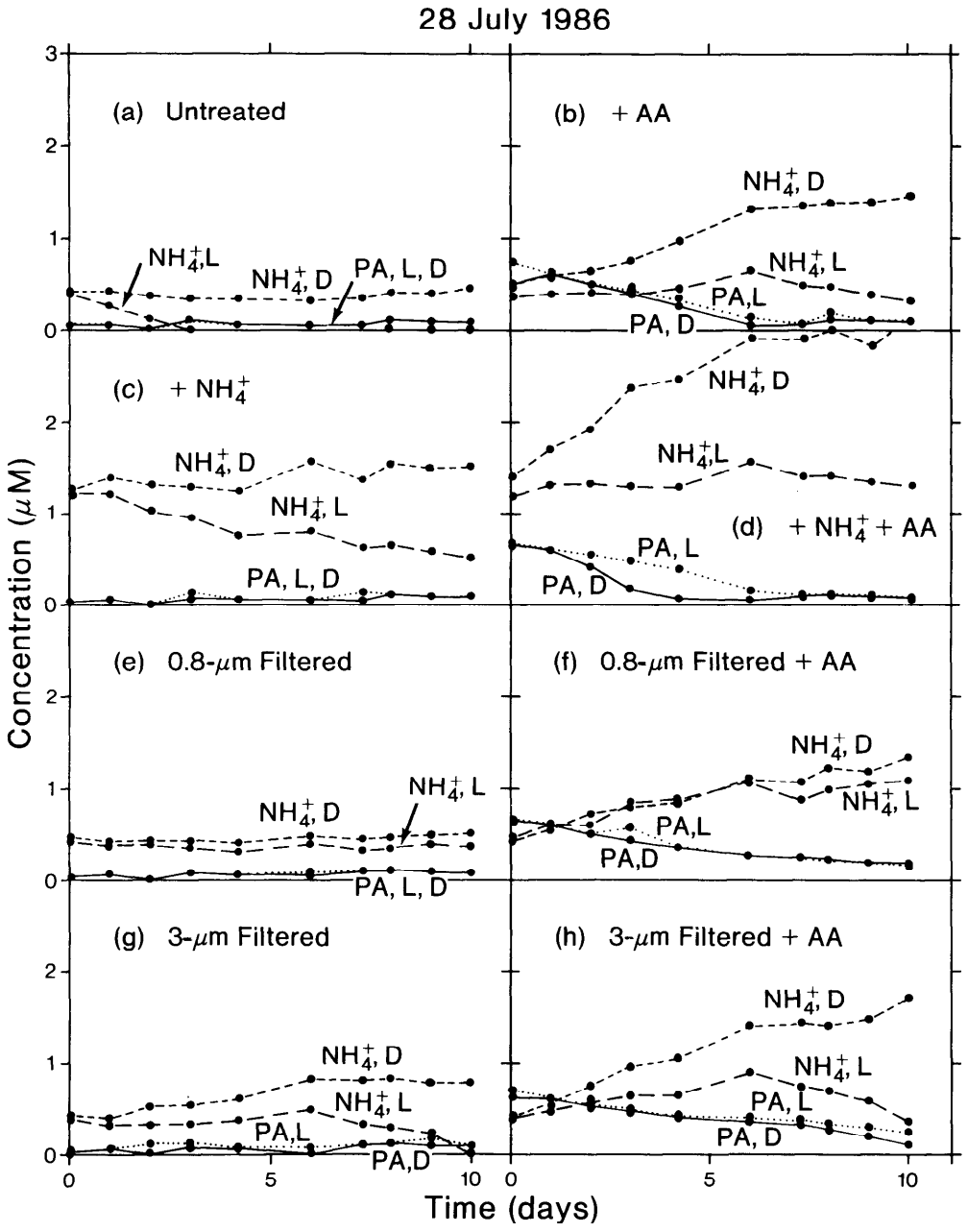

Fig. 2. As Fig. 1, but sampled on 28 July 1986, at in situ temperature of 23°C.

ination during sample handling. A more reasonable explanation may be the selective removal of the effects of mesozooplankter grazer-predators caused by the use of small (70-ml) incubation vessels. Initial concentrations of microbial food-web components would not be affected by the use of small samples, but the mesozooplankton that graze or prey on the algae and protozoans were likely not sampled representatively.

Bacterial abundances did not differ significantly (pairwise t-test $\alpha = 0.05$) between light- and dark-bottle conditions and varied minimally in our experiments. Initial abun-

dances ranged from 6.7 to 11.4 ($\times 10^5$) cells ml^{-1} and resembled numbers previously reported for Lake Michigan (7–10 $\times$ 10^5 cells ml^{-1}: Scavia et al. 1986). Filtration through 3- or 0.8-μm pore-size membranes did not affect bacteria concentrations. Bacterial abundances did not change significantly during incubations for most treatments but did seem to increase slightly (to 8.2–14.9 $\times$ 10^5 cells ml^{-1}) during incubations in the 0.8-μm filtrates fortified with amino acids. The modest increases in bacterial numbers in these filtrates probably resulted both from the removal of potential bacterial grazers and from the addition of a new substrate (DFAA) for the bacteria.

Net amino acid removal rates (slopes of PA concentration vs. time) in light bottles fortified with 0.94 μM DFAA were almost always lower than in corresponding dark bottles (Figs. 1 and 2). These differences can reasonably be attributed to direct, or possibly indirect, amino acid release by metabolizing phytoplankton. Protozoans may also have been involved in the DFAA release process, but we did not specifically examine their role in this study. If bacterial uptake sites are first saturated with amino acids (e.g. at concentrations of $\sim$1 μM for Lake Michigan: Gardner et al. 1986), then bacterial uptake rates of amino acids should be similar in both light/dark and dark bottles, and the differences in net rates of amino acid removal between the two should provide a reliable estimate of amino acid release by phytoplankton. A similar approach with saturating concentrations of inorganic nutrients was used to differentiate phytoplankton uptake from zooplankton release of ammonium and phosphate (Lehman 1980).

Estimates of release of phytoplankton amino acids by this light–dark approach assume that release is greater for photosynthesizing phytoplankton (light/dark conditions) than for phytoplankton held for extended periods in the dark. Our data support this assumption. When we removed the phytoplankton, no significant differences in rates of amino acid uptake were observed between light and dark bottles (i.e. net release in most 0.8-μm pore-size filtrates was not significantly different from zero: Table 1). If selective autolytic release of amino acids by phytoplankton occurs in the dark, then these estimates would be conservative or negative. However, PA concentrations did not increase in any of the dark or light bottles during our incubations; microbial uptake rates apparently balanced any autolytic release of DFAA by old or decaying phytoplankton in the bottles.

The light–dark method allows the estimation of DFAA release, by direct chemical measurement of PA, without removal or inactivation of bacteria in the sample. However, sufficient incubation time is needed for the darkened phytoplankton to first use up stored energy reserves so amino acids and proteins are no longer produced in the same manner as by the lighted phytoplankton (Priscu and Priscu 1984). Care must be exercised in extrapolating such results to the field because this necessary extended incubation can cause changes in phytoplankton content and composition. However, insights gained from these experiments enhance our understanding of nutrient conversion processes in nature.

In our experiments, the changes in PA concentration in the dark were usually almost identical to those in the light during the first 2–3 d. Afterward, the net PA levels in unfiltered samples usually decreased more rapidly in the dark than in the light (e.g. Figs. 1 and 2). Positive slopes with significant regressions for the relationship between amino acid release [$1.46 \times (PA_L - PA_D)$] vs. time were observed for all "untreated" samples and for all but one of those fortified with 1 μM NH_4^+ (Table 1). In contrast, only one of the 0.8-μm filtrates (26 November sample) and three of the 3-μm filtrates (23 April, 7 May, and 23 September samples) exhibited significant release of amino acids.

Rates of amino acid release ranged from undetectable up to 6.3 ng-atoms N $liter^{-1}$ h^{-1} overall and from 0.8 to 3.5 in samples that were not fortified with ammonium or filtered before incubations (Table 1). The high value of 6.3 ng-atoms N $liter^{-1}$ h^{-1} for the NH_4^+-fortified sample of 28 July resulted from an unusual pattern of PA removal and may not be typical. In contrast to other treatments, this sample showed a relatively rapid, exponential decline in ami-

Table 2. Rates of ammonium regeneration (nmol NH_4^+ liter^{-1} h^{-1}) for epilimnetic Lake Michigan water held in the dark for 6–14 d. Rates were calculated as the slope of ammonium accumulation vs. time after 1–2 d were allowed for the phytoplankton to exhaust biochemically stored energy. Number of data sets compared per regression—*n*; correlation coefficient—*r*.

		Untreated		1 μM NH_4^+ addition		3-μm filtrate		0.8-μm filtrate	
1986	*n*	Rate	*r*	Rate	*r*	Rate	*r*	Rate	*r*
23 Apr	9	2.1	0.97	1.9	0.96	2.4	0.87	0.13	0.16
7 May	9	3.0	0.96	4.2	0.90	1.9	0.95	0.37	0.80
22 May	9	0.62	0.91	0.95	0.54	0.80	0.74	0.24	0.50
9 Jun	10	3.6	0.95	2.4	0.87	2.6	0.97	2.4	0.96
9 Jul	4	4.2	0.96	8.7	0.99	2.0	0.98	−0.25	−0.82
28 Jul	9	0.24	0.39	1.0	0.66	1.9	0.90	0.42	0.87
20 Aug	6	3.6	0.96	3.8	0.81	0.46	0.37	0.65	−0.60
23 Sep	5	−1.2	−0.89	0.03	0.02	1.2	0.59	−0.09	−0.26
26 Nov	9	0.42	0.69	0.96	0.92	1.3	0.93	0.19	−0.90

no acids over time (Fig. 2d). Expressed as carbon, our measured rates of phytoplankton amino acid release on untreated samples ranged from 1.1 to 4.8 μg C liter^{-1} d^{-1}. If actual amino acid release in the lake is conservatively assumed to be about a third of that observed in our bottles (based on the increases in phytoplankton biomass observed during containment for the June experiment), amino acid release would be 0.4 to 1.2 μg C liter^{-1} d^{-1}.

These data suggest that release of amino acids by phytoplankton could be an important process supplying organic substrates to bacteria in the lake. Similar conclusions were reached for eutrophic Danish lakes (Jørgensen 1987). Our uncorrected values are about 10% (vs. ~3% for containment-corrected values) of the seasonal range in primary production rates observed at an offshore station (~13–40 μg C liter^{-1} d^{-1}: Scavia et al. 1986). If rates of primary production at our station were approximately double those at the offshore station (based on differences in chlorophyll levels; Moll and Brahce 1986) and if our rates reasonably approximate actual field rates, this comparison suggests that about 5% (or about 2%, with the above containment corrections) of carbon fixed in primary production could be released as DFAA.

Our containment-corrected rates of amino acid C release resemble rates of total PDOC release previously measured by isotopic techniques in fresh Lake Michigan water (0.42–1.54 μg C liter^{-1} d^{-1}: Laird et al. 1986) and in Lake Erie (2.1% of photosynthetic C) or Lake Ontario (4.4% of photosynthetic C) (Lee and Nalewajko 1978). The results from the isotopic studies may have been conservative because of possible incomplete labeling of the algal excretory pools during the relatively short duration (4–26 h) of the experiments (Laird et al. 1986). These data indicate that a significant fraction of PDOC in Lake Michigan could occur as DFAA.

Ammonium accumulation in dark-bottle samples was measured to provide insights about rates of nitrogen mineralization and to allow examination of potential conversions of DFAA-N to NH_4^+-N. These estimates may be biased either by "dark-induced" decomposition of algae or, at the

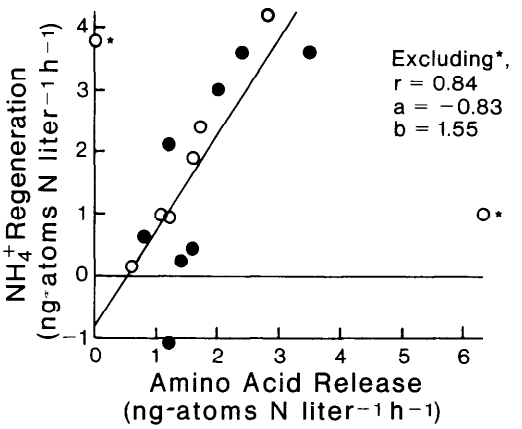

Fig. 3. Comparison of DFAA release to rates of ammonium regeneration for epilimnetic Lake Michigan samples collected in 1986. "Untreated samples"—●; samples treated with 1 μM NH_4^+—○.

Table 3. Rates of ammonium regeneration (nmol NH_4^+ liter^{-1} h^{-1}) for epilimnetic Lake Michigan water fortified with 0.94 μM amino acids and held in the dark for 6–14 d. Rates calculated as in Table 2. Data from time points after the rate of ammonium production declined, due to depletion of added amino acids, were not included in regressions. Number of data sets compared per regression—*n*; correlation coefficient—*r*.

		Untreated		1 μM NH_4^+ addition		3-μm filtrate		0.8-μm filtrate	
1986	*n*	Rate	*r*	Rate	*r*	Rate	*r*	Rate	*r*
7 May	9	5.6	0.95	7.2	0.93	4.2	0.94	1.2	0.79
22 May	9	2.7	0.96	2.2	0.84	1.7	0.91	0.19	0.46
9 Jun	10	6.9	0.99	6.1	0.99	4.1	0.98	3.6	0.98
9 Jul	4	7.7	1.00	13.3	0.98	4.6	1.00	1.8	0.68
28 Jul	6	5.8	0.99	8.4	0.96	6.1	0.99	3.6	0.97
20 Aug	6	7.0	0.97	8.0	0.94	6.7	0.97	6.0	0.96
23 Sep	5	7.4	0.99	4.6	0.95	5.1	0.98	3.3	0.96
26 Nov	9	2.6	0.97	4.0	0.97	3.8	0.99	1.8	0.92

other extreme, by decreased inputs of new labile organic nitrogen due to discontinued photosynthetic activity in the dark bottles, but they are useful for comparison to the amino acid inputs. Except for some initial 1–2-d decreases, ammonium concentrations usually increased in dark bottles. After the initial 1–3-d period, presumably needed for phytoplankton to deplete their energy reserves, approximately linear accumulation of ammonium was usually detected over time in dark-bottle samples (Table 2). In unfiltered samples that were not fortified with amino acids, significant ($P < 0.05$) slopes for rates of dark accumulation of ammonium vs. time were observed for all but the 28 July sample (Table 2). Rates of dark accumulation of ammonium in the untreated samples ranged from −1.2 to 4.2 nmol NH_4^+ liter^{-1} h^{-1} (Table 2). Similar rates were observed in the samples that were fortified with ammonium or passed through 3-μm pore-size filters, but some slopes were not significant.

In contrast, ammonium levels decreased or remained at background levels in light bottles (Fig. 1), except for some PA-fortified samples in which NH_4^+ production exceeded removal by phytoplankton (Fig. 2b, d, f, and h). Rates of ammonium regeneration were low (slopes were often not significantly different from zero) in the 0.8-μm filtrates except for the 9 June filtrate that yielded a rate comparable to the unfiltered samples (Table 2). These low rates could indicate that micrograzers, rather than bacteria, were the primary mineralizers in the unfiltered water (e.g. Goldman et al. 1985) or possibly that the ammonium accumulation in the unfiltered water (or the 3-μm filtrates) resulted mainly from the decomposition of particles (e.g. dead phytoplankton) in the dark bottles.

Rates of dark ammonium production in bottles of unfiltered Lake Michigan water correlated significantly ($P < 0.01$) with rates of amino acid release, by the relationship, "NH_4^+-N production = $-0.83 + (1.55) \times$ (amino-acid-N release)" ($r = 0.84$; $n = 14$) if two outliers (July and August samples with ammonium additions) were excluded (Fig. 3). On average, the rates of ammonium production were higher than those of amino acid release, probably due to the recycling of dissolved or particulate labile organic nitrogen occurring in forms other than DFAA (e.g. proteins or peptides).

Addition of amino acids to Lake Michigan water always enhanced the rates and amounts of ammonium accumulation in the dark (Tables 3 vs. 2). When amino acids were added, significant regeneration of ammonium was observed for all the experimental treatments except for two 0.8-μm pore-size filtrates (Table 3). Rates for unfiltered samples ranged from 2 to 13 nmol NH_4^+ liter^{-1} h^{-1} and correlation coefficients for ammonium accumulation vs. time were all >0.84 (Table 3).

By comparing the net differences in ammonium production in dark bottles caused by addition of amino acids with corresponding decreases in DFAA-N in fortified samples over the same period, we could estimate the percentage of assimilated amino acid nitrogen that was mineralized vs. in-

corporated into food-web biomass. If corrections were made for natural ammonium production (i.e. rates in unfortified dark bottles), increased ammonium production in samples fortified with amino acids was related to amino acid removal over the same period. The average ratio of increased ammonium-N produced to DFAA-N removed for our samples was 1.01 (SE = 0.08, n = 29). Thus, on average, the amount of DFAA-N removed from solution was equal to the amount that appeared as increased ammonium in the same bottles. It implies that the added amino acids likely were quantitatively deaminated and that little of the nitrogen in our bottles was incorporated into food-web biomass. (It also indicates that the ammonium produced in water was not nitrified in our experimental systems.)

Although the dynamics of carbon and nitrogen are not strictly comparable, the idea that most DFAA-N is not incorporated into "upper food-web" biomass agrees in principle with the concept that the microbial food web functions primarily as a sink for carbon in planktonic food webs and that this role of mineralizer is more important than the role of assimilator of organic material into higher trophic levels (Ducklow et al. 1986) in Lake Michigan. The apparently complete deamination of the added amino acids that were removed from solution was somewhat surprising because the use of amino acid carbon for energy, with the associated energy-cost of deamination, seems less efficient than the use of the compounds directly for producing biomass (proteins). For example, amino acids are a less efficient substrate for production of aquatic bacteria than is a combination of glucose + ammonium (Wheeler and Kirchman 1986).

Although it is speculation, the apparent quantitative deamination of added amino acids that we observed may imply a scarcity of other sources of organic carbon energy that are readily available to heterotrophs in epilimnetic Lake Michigan water. Amino acids may constitute a relatively high percentage of new, low-molecular-weight organic carbon released by phytoplankton (Mague et al. 1980; Jørgensen 1987). This scenario may be reasonable for epilimnetic Lake Michigan water where the phytoplankton are not limited by inorganic nitrogen (Bartone and Schelske 1982) or light (Fahnenstiel and Scavia 1987*a*).

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